FavorPrep™
96-well Genomic DNA Kit

User Manual

Cat. No.: FADWE 000 (1 plate)
FADWE 001 (4 plates)
FADWE 002 (10 plates)

For Research Use Only
v.1307

Introduction

96-Well Genomic DNA Extraction Kit is designed for high-throughput purification of total DNA (including genomic, mitochondrial and viral DNA) from whole blood and a variety of animal tissues or cells. The method use proteinase K and a chaotropic salt, guanidine hydrochloride to lyse cells and degrade protein, then DNA in chaotropic salt is bonded to glass fiber matrix of plate. After washing off the contaminants, the purified DNA is eluted by low salt elution buffer or water. The entire procedure can be completed in one hour without phenol/ chloroform extraction and alcohol precipitation. The kits can be used for manual filtration or with robotic handing systems and purified DNA with approximately 20-30 kb is suitable for PCR or other enzymatic reactions.

Quality Control

The quality of 96-Well Genomic DNA Kit is tested on a lot-to-lot basis. The purified DNA is checked byagarose gel analysis and quantified with spectrophotometer.

Kit Content

	FADWE000 1 plate	FADWE001 4 plates	FADWE002 10 plates
FATG1 Buffer	23 ml	120 ml	250 ml
FATG2 Buffer	23 ml	120 ml	250 ml
W1 Buffer (concentrated)	33 ml **	95 ml **	95 ml ** x 3
Wash Buffer (concentrated)	20 ml *	50 ml *	50 ml * x 3
Elution Buffer	23 ml	60 ml	240 ml
Proteinase K	23 mg †	90 mg ††	225 mg †††
96-Well DNA binding plate	1 pcs	4 pcs	10 pcs
96-Well PCR plate	1 pcs	4 pcs	10 pcs
Adhesive Film	2 pcs	8 pcs	20 pcs

*** Add 100 / 200ml of ethanol (96-100%) to each Wash Buffer when first use.**
**** Add 12 / 35 ml of ethanol (96-100%) to each W1 Buffer when first use.**
† Add 2.3 ml of ddH_2O to the bottle and mix well, store the prepared proteinase K at 4 °C.
†† Add 9 ml of ddH_2O to the bottle and mix well, store the prepared proteinase K at 4 °C.
††† Add 22.5 ml of ddH_2O to the bottle and mix well, store the prepared proteinase K at 4 °C.

Specification

Sample: up to 200 µl of fresh/ frozen blood per well
up to 25 mg of animal tissue
up to 5 X 10^7 animal cultured cells
up to 10^8 bacterial cultured cells

Binding Capacity: up to 30 µg/ well

Elution Volume: 50-100 µl

Operation: centrifuge/ vacuum & centrifuge

Handling Time: within 90 minutes

Important Note

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.

2. The maxium sample size is described on Specification, do not use the sample more than the limitation.

3. Add required volume of ethanol (96- 100 %) to Wash Buffer when first open.

4. Add requred volume of ddH_2O to proteinase K to prepare the 10 mg/ml proteinase K solution and store the solution at 4 °C.

5. Prepare two dry baths or two water baths to 60 °C and 70 °C before the operation.

6. Preheat the Elution Buffer to 65 °C for DNA elution.

Additional Requirements:

1. 96-well 2.0 ml plate (2.0 ml, 96 well deep collection plate)

2. Centrifuge equiment with a swing-bucket rotor, capable of at least 5,000 X g

3. 65 °C and 70 °C waterbaths or dry baths

4. Absolute (96~100%) ethanol

Centrifuge Protocol for Blood Sample

Please Read Important Notes Before Starting The Following Steps.

Step 1
Cell lysis

- Add 200 µl FATG2 Buffer and 20 µl Proteinase K (10 mg/ ml) to each well of a 96-Well 2 ml plate (not provided).
- Apply 200 µl of blood sample to each well and mix by pipetting. Seal with Adhesive Film.
- Incubate at 60 °C for 20 minutes.
- Preheat required Elution Buffer (50~100 µl per well) at 60 °C. (For Step 4 DNA elution)

Add FATG2 Buffer and Proteinase K

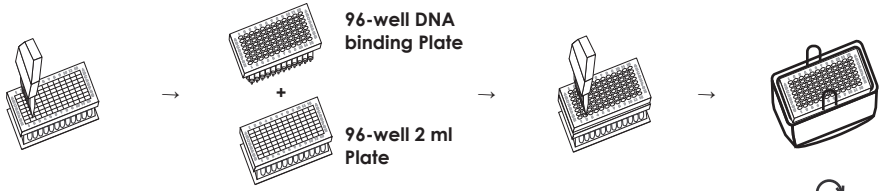
Add Blood sample and mix by pipetting

Seal with adhesive film

Incubate at 60 °C for 20 minutes

STEP 2
DNA Binding

- Add 200 µl ethanol (96~100%) to each well of sample lysate in 96-well 2ml plate from precious step. Mix immediately by pipetting 5-10 times.
- Place a 96-Well DNA binding Plate on top of another 96-Well 2 ml Plate (not provided).
- Transfer each well of the sample mixture to the 96-Well DNA binding Plate.
- Place the assembly plates (96-Well DNA binding Plate + 96-Well 2 ml plate) in a rotor bucket and centrifuge at 4,500 – 6,000 x g for 5 min.
- Discard the flow-through and return the 96-DNA binding Plate on top of the 96-Well 2 ml Plate.



Add ethanol and mix by pipetting

Assemble plates

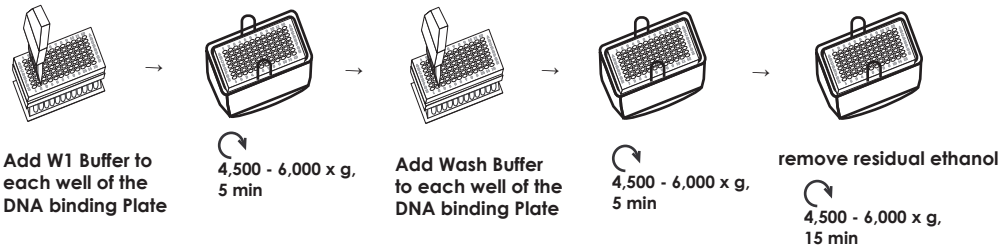
Transfer the sample mixture to the assemble plates

4,500 - 6,000 x g, 10 min

STEP 3

Washing

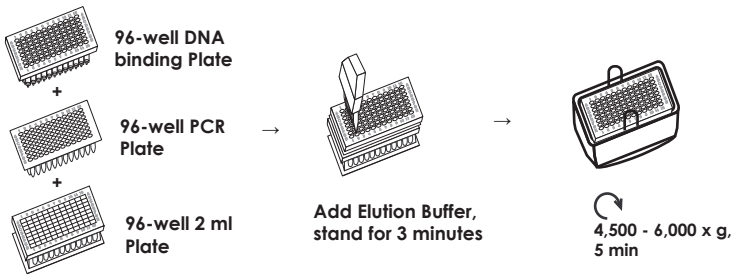
- **Add 300 µl W1 Buffer to each well of the 96-Well DNA binding Plate.**
- **Place the assembly plates in a rotor bucket and centrifuge at 4,500 – 6,000 x g for 5 min.**
- **Discard the flow-through and return the 96-Well DNA binding Plate on top of the 96-Well 2 ml Plate.**
- **Add 600 µl Wash Buffer (ethanol added) to each well of the 96-Well DNA binding Plate.**
- **Place the assembly plates in a rotor bucket and centrifuge at 4,500 – 6,000 x g for 5 min.**
- **Discard the flow-through and return the 96-Well DNA binding Plate on top of the 96-Well 2 ml Plate.**
- **Place the assembly plates in a rotor bucket and centrifuge at 4,500 – 6,000 x g for an additional 15 minutes to remove residual ethanol.**



STEP 4

DNA Elution

- **Place a clean 96-well PCR Plate (provided) on top of the 96-Well 2 ml Plate. And place the 96-Well DNA binding Plate on the clean 96-Well PCR plate.**
  (top: 96-well DNA binding Plate, middle: 96-well PCR Plate, bottom: 96-Well 2 ml plate)
- **Add 50~100 µl Elution Buffer or ddH$_2$O (pH8.0-8.5) into the membrane center of the 96-Well DNA binding Plate. Stand for 3 minutes until Elution Buffer or ddH2O has been absorbed by the membrane completely.**
- **Place the assembly plates in a rotor bucket and centrifuge for 5 min at 4,500 – 6,000 x g to elute purified DNA.**



Vaccum/Centrifuge Protocol for Blood Sample

Please Read Important Notes Before Starting The Following Steps.

Step 1
Cell lysis

- Add 200 µl FATG2 Buffer and 20 µl Proteinase K (10 mg/ ml) to each well of a 96-Well 2 ml plate (not provided).
- Apply 200 µl of blood sample to each well and mix by pipetting. Seal with Adhesive Film.
- Incubate at 60 °C for 20 minutes.
- Preheat required Elution Buffer (50~100 µl per well) at 60 °C. (For Step 4 DNA elution)

Add FATG2 Buffer and Proteinase K

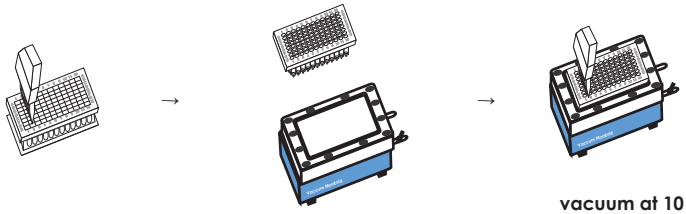
Add Blood sample and mix by pipetting

Seal with adhesive film

Incubate at 60 °C for 20 minutes

STEP 2
DNA Binding

- Add 200 µl ethanol (96~100%) to each well of sample lysate in 96-well 2ml plate from precious step. Mix immediately by pipetting 5-10 times.
- Place a 96-Well DNA binding Plate on top of the vacuum manifold.
- Transfer each well of the sample mixture to the 96-Well DNA binding Plate.
- Apply vacuum at 10 inches Hg for 5 minutes until wells have emptied.
- Discard the flow-through and return the 96-DNA binding Plate on top of the 96-Well 2 ml Plate.



Add ethanol and mix by pipetting

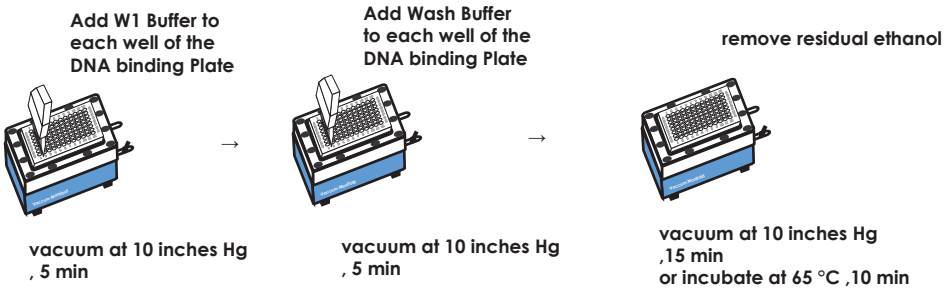
Transfer the sample mixture to the assemble plates

vacuum at 10 inches Hg , 5 min

STEP 3

Washing

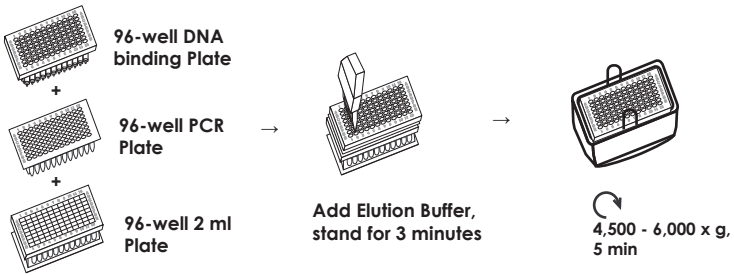
- **Add 300 µl W1 Buffer to each well of the 96-Well DNA binding Plate.**
- **Apply vacuum at 10 inches Hg for 5 minutes until wells have emptied.**
- **Add 600 µl Wash Buffer (ethanol added) to each well of the 96-Well DNA binding Plate.**
- **Apply vacuum at 10 inches Hg for 5 minutes until wells have emptied.**
- **Apply vacuum at 10 inches Hg for an additional 15 min or incubate at 65°C for 10 min to remove residual ethanol.**



STEP 4

DNA Elution

- **Place a clean 96-well PCR Plate (provided) on top of the 96-Well 2 ml Plate. And place the 96-Well DNA binding Plate on the clean 96-Well PCR plate.**
  (top: 96-well DNA binding Plate, middle: 96-well PCR Plate, bottom: 96-Well 2 ml plate)
- **Add 50~100 µl Elution Buffer or ddH$_2$O (pH8.0-8.5) into the membrane center of the 96-Well DNA binding Plate. Stand for 3 minutes until Elution Buffer or ddH2O has been absorbed by the membrane completely.**
- **Place the assembly plates in a rotor bucket and centrifuge for 5 min at 4,500 – 6,000 x g to elute purified DNA.**

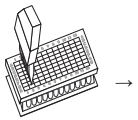


Centrifuge Protocol for Tissue Sample

Please Read Important Notes Before Starting The Following Steps.

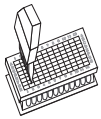
Step 1
Cell lysis

- Add 200 µl FATG1 Buffer and 20 µl Proteinase K (10 mg/ ml) to each well of a 96-Well 2 ml plate (not provided).
- Cut up to 25 mg of animal tissues (or 0.5 cm of mouse tail) and transfer into each well of 96-Well 2 ml plate. Seal with Adhesive Film.
- Incubate the plate with shaking at 60°C for 1-2 hours to lyse the sample.
- If RNA-free genomic DNA is required, add 5 µl of RNase A (50 mg/ ml, not provided) to each well and incubate at room temperature for 4 minutes.
- Add 200 µl FATG2 Buffer to each well and mix by shaking.
- Seal with Adhesive Film. Incubate the plate with shaking at 70 °C for 20 minutes until the sample lysate is clear.
- Preheat required Elution Buffer (50~100µl per sample) at 70 °C. (For Step 4 DNA elution)
- If there are insoluble material present following incubation, centrifuge the plate for 5 minutes at full speed and transfer the supernatants to a new 96-Well 2 ml plate (not provided).



Add FATG1 Buffer and Proteinase K

→ Transfer tissue to each well of the 2 ml plate, Seal with adhesive film. → Incubate the plate with shaking at 60 °C for 1-2 hrs



Add FATG2 Buffer. Seal with adhesive film, mix by shaking

→ Incubate the 2 ml plate with shaking at 70 °C for 20 minutes

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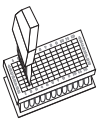
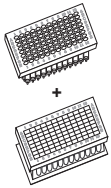
STEP 2
DNA Binding

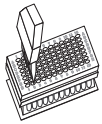
- Add 200 µl ethanol (96~100%) to each well of sample lysate in 96-well 2ml plate from precious step. Mix immediately by pipetting 5-10 times.
- Place a 96-Well DNA binding Plate on top of another 96-Well 2 ml Plate (not provided).
- Transfer each well of the sample mixture to the 96-Well DNA binding Plate.
- Place the assembly plates (96-Well DNA binding Plate + 96-Well 2 ml plate) in a rotor bucket and centrifuge at 4,500 – 6,000 x g for 5 min.
- Discard the flow-through and return the 96-DNA binding Plate on top of the 96-Well 2 ml Plate.



Add ethanol and mix by pipetting

→ 

Assemble plates

→ 

Transfer the sample mixture to the assemble plates

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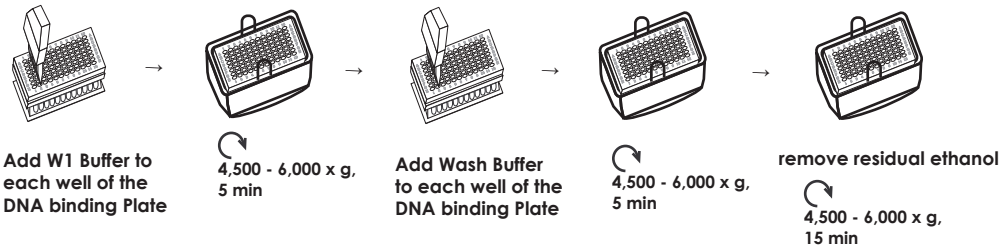
4,500 - 6,000 x g, 10 min

STEP 3

Washing

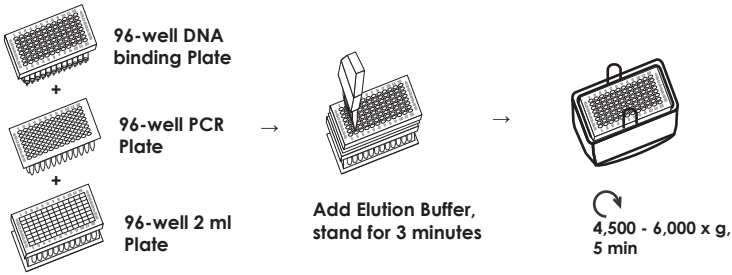
- **Add 300 µl W1 Buffer to each well of the 96-Well DNA binding Plate.**
- **Place the assembly plates in a rotor bucket and centrifuge at 4,500 – 6,000 x g for 5 min.**
- **Discard the flow-through and return the 96-Well DNA binding Plate on top of the 96-Well 2 ml Plate.**
- **Add 600 µl Wash Buffer (ethanol added) to each well of the 96-Well DNA binding Plate.**
- **Place the assembly plates in a rotor bucket and centrifuge at 4,500 – 6,000 x g for 5 min.**
- **Discard the flow-through and return the 96-Well DNA binding Plate on top of the 96-Well 2 ml Plate.**
- **Place the assembly plates in a rotor bucket and centrifuge at 4,500 – 6,000 x g for an additional 15 minutes to remove residual ethanol.**



STEP 4

DNA Elution

- **Place a clean 96-well PCR Plate (provided) on top of the 96-Well 2 ml Plate. And place the 96-Well DNA binding Plate on the clean 96-Well PCR plate.**
  (top: 96-well DNA binding Plate, middle: 96-well PCR Plate, bottom: 96-Well 2 ml plate)
- **Add 50~100 µl Elution Buffer or ddH$_2$O (pH8.0-8.5) into the membrane center of the 96-Well DNA binding Plate. Stand for 3 minutes until Elution Buffer or ddH2O has been absorbed by the membrane completely.**
- **Place the assembly plates in a rotor bucket and centrifuge for 5 min at 4,500 – 6,000 x g to elute purified DNA.**

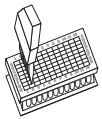


Vaccum/Centrifuge Protocol for Tissue Sample

Please Read Important Notes Before Starting The Following Steps.

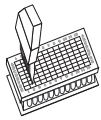
Step 1
Cell lysis

- Add 200 µl FATG1 Buffer and 20 µl Proteinase K (10 mg/ ml) to each well of a 96-Well 2 ml plate (not provided).
- Cut up to 25 mg of animal tissues (or 0.5 cm of mouse tail) and transfer into each well of 96-Well 2 ml plate. Seal with Adhesive Film.
- Incubate the plate with shaking at 60°C for 1-2 hours to lyse the sample.
- If RNA-free genomic DNA is required, add 5 µl of RNase A (50 mg/ ml, not provided) to each well and incubate at room temperature for 4 minutes.
- Add 200 µl FATG2 Buffer to each well and mix by shaking.
- Seal with Adhesive Film. Incubate the plate with shaking at 70 °C for 20 minutes until the sample lysate is clear.
- Preheat required Elution Buffer (50~100µl per sample) at 70 °C. (For Step 4 DNA elution)
- If there are insoluble material present following incubation, centrifuge the plate for 5 minutes at full speed and transfer the supernatants to a new 96-Well 2 ml plate (not provided).



Add FATG1 Buffer and Proteinase K

→ Transfer tissue to each well of the 2 ml plate, Seal with adhesive film. → Incubate the plate with shaking at 60 °C for 1-2 hrs



Add FATG2 Buffer. Seal with adhesive film, mix by shaking

→ Incubate the 2 ml plate with shaking at 70 °C for 20 minutes

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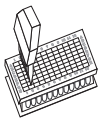
STEP 2
DNA Binding

- Add 200 µl ethanol (96~100%) to each well of sample lysate in 96-well 2ml plate from precious step. Mix immediately by pipetting 5-10 times.
- Place a 96-Well DNA binding Plate on top of the vacuum manifold.
- Transfer each well of the sample mixture to the 96-Well DNA binding Plate.
- Apply vacuum at 10 inches Hg for 5 minutes until wells have emptied.
- Discard the flow-through and return the 96-DNA binding Plate on top of the 96-Well 2 ml Plate.



Add ethanol and mix by pipetting

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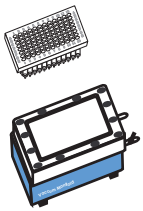

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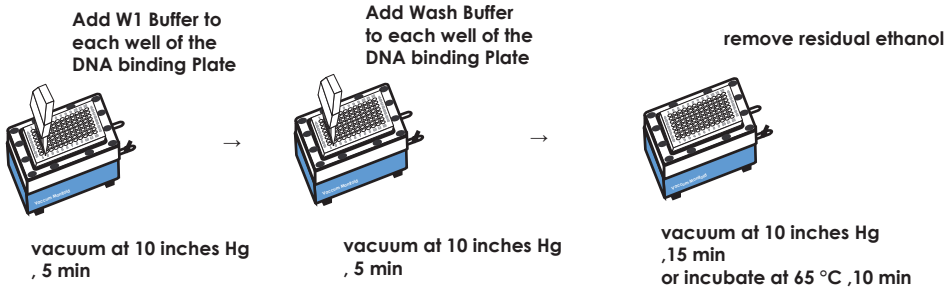
vacuum at 10 inches Hg , 5 min

Transfer the sample mixture to the assemble plates

STEP 3

Washing

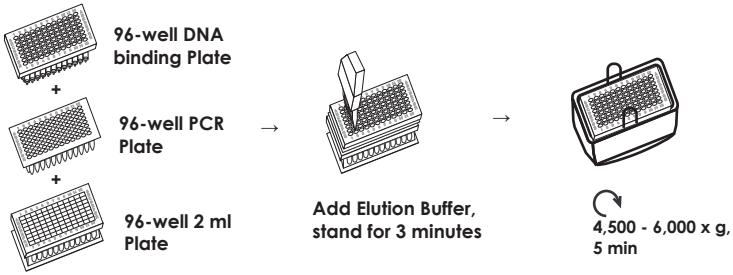
- **Add 300 µl W1 Buffer to each well of the 96-Well DNA binding Plate.**
- **Apply vacuum at 10 inches Hg for 5 minutes until wells have emptied.**
- **Add 600 µl Wash Buffer (ethanol added) to each well of the 96-Well DNA binding Plate.**
- **Apply vacuum at 10 inches Hg for 5 minutes until wells have emptied.**
- **Apply vacuum at 10 inches Hg for an additional 15 min or incubate at 65ºC for 10 min to remove residual ethanol.**



STEP 4

DNA Elution

- **Place a clean 96-well PCR Plate (provided) on top of the 96-Well 2 ml Plate. And place the 96-Well DNA binding Plate on the clean 96-Well PCR plate.**
  (top: 96-well DNA binding Plate, middle: 96-well PCR Plate, bottom: 96-Well 2 ml plate)
- **Add 50~100 µl Elution Buffer or ddH$_2$O (pH8.0-8.5) into the membrane center of the 96-Well DNA binding Plate. Stand for 3 minutes until Elution Buffer or ddH2O has been absorbed by the membrane completely.**
- **Place the assembly plates in a rotor bucket and centrifuge for 5 min at 4,500 – 6,000 x g to elute purified DNA.**



Centrifuge Protocol for Cultured Cells Sample

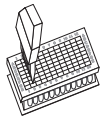
Please Read Important Notes Before Starting The Following Steps.

Step 1
Cell Harvesting

- Transfer the cultured cells to each well of a 96-Well 2 ml Plate (not provided).
- Centrifuge at 1,000 x g for 10 minutes to pellet the cells, discard the supernatant.

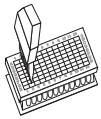
Step 2
Cell lysis

- Add 200 µl FATG1 Buffer and 20 µl Proteinase K (10 mg/ ml) to each well of the 96-Well 2 ml plate and resuspend the pellet by pipetting.
- Seal with adhesive film and incubate the plate with shaking at 60°C for 20 min to lyse the sample.
- If RNA-free genomic DNA is required, add 5 µl of RNase A (50 mg/ ml, not provided) to each well and incubate at room temperature for 4 minutes.
- Add 200 µl FATG2 Buffer to each well. Seal with Adhesive Film and mix by shaking.
- Incubate the plate with shaking at 70 °C for 20 minutes until the sample lysate is clear.
- Preheat required Elution Buffer (50~100 µl per well) at 70 °C. (For Step 5 DNA elution)



Add FATG1 Buffer and Proteinase K, Resuspend the pellet by pipetting

→ Seal with adhesive film. Incubate the 2 ml plate with shaking at 60 °C for 20 min



Add FATG2 Buffer. Seal with adhesive film, mix by shaking

→ Incubate the 2 ml plate with shaking at 70 °C for 20 minutes

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STEP 3
DNA Binding

- Add 200 µl ethanol (96~100%) to each well of sample lysate in 96-well 2ml plate from precious step. Mix immediately by pipetting 5-10 times.
- Place a 96-Well DNA binding Plate on top of another 96-Well 2 ml Plate (not provided).
- Transfer each well of the sample mixture to the 96-Well DNA binding Plate.
- Place the assembly plates (96-Well DNA binding Plate + 96-Well 2 ml plate) in a rotor bucket and centrifuge at 4,500 – 6,000 x g for 5 min.
- Discard the flow-through and return the 96-DNA binding Plate on top of the 96-Well 2 ml Plate.

Add ethanol and mix by pipetting

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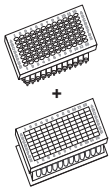

Assemble plates

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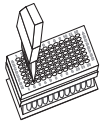

Transfer the sample mixture to the assemble plates

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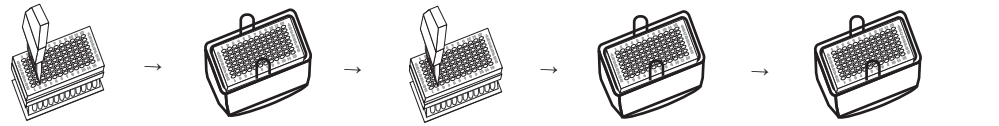
4,500 - 6,000 x g, 10 min

STEP 4

Washing

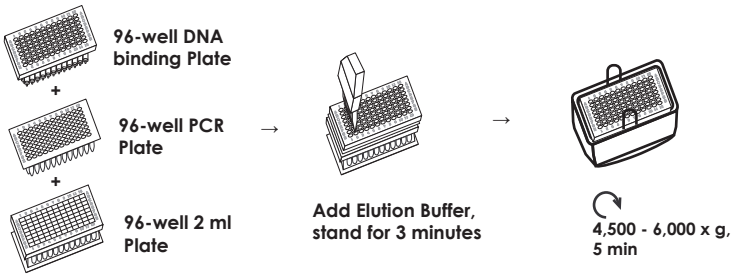
- **Add 300 µl W1 Buffer to each well of the 96-Well DNA binding Plate.**
- **Place the assembly plates in a rotor bucket and centrifuge at 4,500 – 6,000 x g for 5 min.**
- **Discard the flow-through and return the 96-Well DNA binding Plate on top of the 96-Well 2 ml Plate.**
- **Add 600 µl Wash Buffer (ethanol added) to each well of the 96-Well DNA binding Plate.**
- **Place the assembly plates in a rotor bucket and centrifuge at 4,500 – 6,000 x g for 5 min.**
- **Discard the flow-through and return the 96-Well DNA binding Plate on top of the 96-Well 2 ml Plate.**
- **Place the assembly plates in a rotor bucket and centrifuge at 4,500 – 6,000 x g for an additional 15 minutes to remove residual ethanol.**



STEP 5

DNA Elution

- **Place a clean 96-well PCR Plate (provided) on top of the 96-Well 2 ml Plate. And place the 96-Well DNA binding Plate on the clean 96-Well PCR plate.**
  (top: 96-well DNA binding Plate, middle: 96-well PCR Plate, bottom: 96-Well 2 ml plate)
- **Add 50~100 µl Elution Buffer or ddH$_2$O (pH8.0-8.5) into the membrane center of the 96-Well DNA binding Plate. Stand for 3 minutes until Elution Buffer or ddH2O has been absorbed by the membrane completely.**
- **Place the assembly plates in a rotor bucket and centrifuge for 5 min at 4,500 – 6,000 x g to elute purified DNA.**



Vaccum/Centrifuge Protocol for Cultured Cells Sample

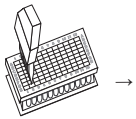
Please Read Important Notes Before Starting The Following Steps.

Step 1
Cell Harvesting

- Transfer the cultured cells to each well of a 96-Well 2 ml Plate (not provided).
- Centrifuge at 1,000 x g for 10 minutes to pellet the cells, discard the supernatant.

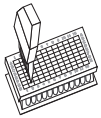
Step 2
Cell lysis

- Add 200 µl FATG1 Buffer and 20 µl Proteinase K (10 mg/ ml) to each well of the 96-Well 2 ml plate and resuspend the pellet by pipetting.
- Seal with adhesive film and incubate the plate with shaking at 60°C for 20 min to lyse the sample.
- If RNA-free genomic DNA is required, add 5 µl of RNase A (50 mg/ ml, not provided) to each well and incubate at room temperature for 4 minutes.
- Add 200 µl FATG2 Buffer to each well. Seal with Adhesive Film and mix by shaking.
- Incubate the plate with shaking at 70 °C for 20 minutes until the sample lysate is clear.
- Preheat required Elution Buffer (50~100 µl per well) at 70 °C. (For Step 5 DNA elution)



Add FATG1 Buffer and Proteinase K, Resuspend the pellet by pipetting

→ Seal with adhesive film. Incubate the 2 ml plate with shaking at 60 °C for 20 min



Add FATG2 Buffer. Seal with adhesive film, mix by shaking

→ Incubate the 2 ml plate with shaking at 70 °C for 20 minutes

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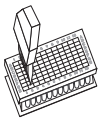
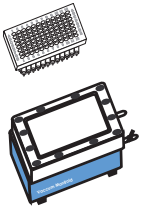
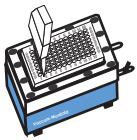
STEP 3
DNA Binding

- Add 200 µl ethanol (96~100%) to each well of sample lysate in 96-well 2ml plate from precious step. Mix immediately by pipetting 5-10 times.
- Place a 96-Well DNA binding Plate on top of the vacuum manifold.
- Transfer each well of the sample mixture to the 96-Well DNA binding Plate.
- Apply vacuum at 10 inches Hg for 5 minutes until wells have emptied.
- Discard the flow-through and return the 96-DNA binding Plate on top of the 96-Well 2 ml Plate.



Add ethanol and mix by pipetting





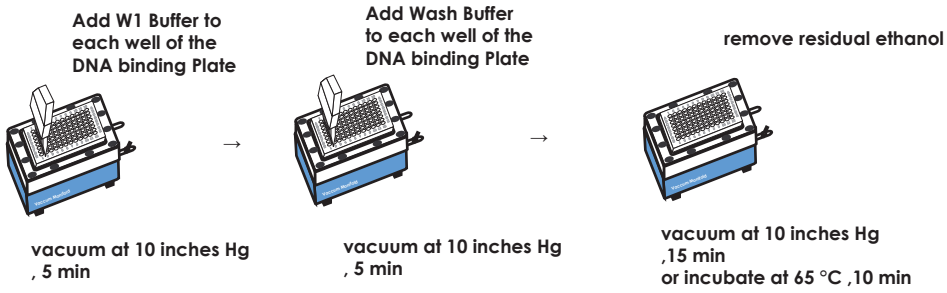
vacuum at 10 inches Hg , 5 min

Transfer the sample mixture to the assemble plates

STEP 4

Washing

- **Add 300 µl W1 Buffer to each well of the 96-Well DNA binding Plate.**
- **Apply vacuum at 10 inches Hg for 5 minutes until wells have emptied.**
- **Add 600 µl Wash Buffer (ethanol added) to each well of the 96-Well DNA binding Plate.**
- **Apply vacuum at 10 inches Hg for 5 minutes until wells have emptied.**
- **Apply vacuum at 10 inches Hg for an additional 15 min or incubate at 65°C for 10 min to remove residual ethanol.**



STEP 5

DNA Elution

- **Place a clean 96-well PCR Plate (provided) on top of the 96-Well 2 ml Plate. And place the 96-Well DNA binding Plate on the clean 96-Well PCR plate.**
  (top: 96-well DNA binding Plate, middle: 96-well PCR Plate, bottom: 96-Well 2 ml plate)
- **Add 50~100 µl Elution Buffer or ddH$_2$O (pH8.0-8.5) into the membrane center of the 96-Well DNA binding Plate. Stand for 3 minutes until Elution Buffer or ddH2O has been absorbed by the membrane completely.**
- **Place the assembly plates in a rotor bucket and centrifuge for 5 min at 4,500 – 6,000 x g to elute purified DNA.**